American Journal of Medical Genetics (Neuropsychiatric Genetics) 74:150–153 (1997)

Lack of Association Between Juvenile Myoclonic Epilepsy and GABRA5 and GABRB3 Genes

Michel Guipponi,[1]* Pierre Thomas,[2] Claire Girard-Reydet,[1] Josué Feingold,[3] Michel Baldy-Moulinier,[1] and Alain Malafosse[1,4]

[1]*Laboratoire de Médecine Expérimentale, Institut de Biologie, INSERM U 249-CNRS UPR 9008, Montpellier, France*
[2]*Service de Neurologie, Hôpital Pasteur, Nice, France*
[3]*INSERM U 155 Epidémiologie Génétique, Château de Longchamp-Bois de Boulogne, Paris, France*
[4]*Division de Neuropsychiatrie, IUPG, Chêne-Bourg, Switzerland*

Alpha5 and beta3 $GABA_A$ receptor genes are major candidates for epilepsy, as they code for subunits of the most important human inhibitory neurotransmitter. Moreover, they are located within a region of the human genome previously implicated in disorders including epilepsy. We carried out an association study between dinucleotide repeat polymorphisms in these two genes and juvenile myoclonic epilepsy (JME). JME is the most common idiopathic epilepsy and is characterized by a complex mode of inheritance. We did not find significant differences between controls and patients for allele or genotype frequencies. Am. J. Med. Genet. 74:150–153, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: juvenile myoclonic epilepsy; $GABA_A$ receptor subunit genes; association study

INTRODUCTION

Juvenile myoclonic epilepsy (JME) is a common form of adolescent-onset idiopathic generalized epilepsy (IGE) comprising about 8% of all epilepsies. JME is characterized by myoclonic jerks, mainly on awakening and often associated with generalized tonic-clonic seizures (GTCS) and typical absence seizures [Delgado-Escueta and Enrile Bascal, 1984]. Ictal EEG is characterized by high-amplitude multispikes followed by slow waves during myoclonus. Interictal EEGs show 3–6 Hz generalized multispike-wave complexes [Grunewald and Panayiotopoulos, 1993]. Family and twin studies implicate genetic factors in the etiology of JME [Janz et al., 1989; Delgado-Escueta et al., 1990; Anderson et al., 1990], although the mode of inheritance remains unknown. Polygenic [Tsuboi and Christian, 1973], multifactorial, autosomal-dominant [Delgado-Escueta et al., 1990], autosomal-recessive [Panayiotopoulos and Obeid 1989], and digenic [Greenberg et al., 1988a] modes of inheritance have all been proposed.

Greenberg et al. [1988b] reported evidence for linkage between JME and the HLA region on chromosome 6p. Lod scores were maximized if clinically symptomatic family members with paroxysmal EEG abnormalities were classified as affected. Weissbecker et al. [1991] and Durner et al. [1991] also reported linkage between JME and HLA (using both serological and DNA markers) in an independent set of pedigrees. However, the highest lod score was obtained when EEG abnormalities in asymptomatic individuals were not taken into account [Weissbecker et al., 1991].

Linkage of JME to the HLA region was not found in other pedigrees of different origins [Liu et al., 1992; Whitehouse et al., 1993; Elmslie et al., 1995]. Genetic heterogeneity may explain these discrepancies. However, differences between the ways in which these pedigrees were ascertained and their small size did not allow a test of this possibility. Another explanation is that positive lod scores were obtained in some pedigrees because of multiple testing. Indeed, it is known that testing several clinical and genetic models increases the risk of obtaining false evidence for linkage [Clerget-Darpoux et al., 1990].

Consequently, there is to date no firm evidence for a major gene (i.e., with a Mendelian mode of inheritance) determining JME. Therefore, the exclusive use of the lod score method, which is dependent on a knowledge of the genetic parameters of the disease under study, is not appropriate. For such diseases with a complex mode of inheritance, the combination of both parametric and nonparametric methods is now recommended. For Risch and Merikangas [1996], the future of the genetics of complex diseases lies in the use of association analysis.

*Correspondence to: Michel Guipponi, Laboratoire de Médecine Expérimentale, Institut de Biologie, INSERM U 249-CNRS UPR 9008, Boulevard Henri IV, 34060 Montpellier, France.

Received 21 May 1996; Revised 13 December 1996

As part of a genetic study of JME, we present here the results of an association study with two candidate genes: the alpha5 and beta3 subunits of the γ-aminobutyric acid A ($GABA_A$) receptor (GABRA5 and GABRB3, respectively). GABA is the principal inhibitory neurotransmitter in the mammalian central nervous system, and several lines of evidence implicate it in the pathophysiology of epilepsies [Meldrum, 1975]. Moreover, the GABRA5 and GABRB3 genes have been mapped to chromosome 15q11–q13. The locus containing these two genes encompasses 400 kb [Buckle et al., 1989; Sinnett et al., 1993]. Recently, two different studies reported patients with epilepsy associated in one case with Prader-Willi syndrome and in the other case with autism and ataxia [Bundey et al., 1994; Aguglia et al., 1995]. Molecular genetic studies showed a duplication-inversion of chromosome 15q11–q13 in the first case [Bundey et al., 1994] and a duplication of the GABRA5 and GABRB3 genes in the second [Aguglia et al., 1995].

PATIENTS AND METHODS

JME Patients

Patients with JME were randomly selected from neurology departments of five different hospitals, independent of the presence or absence of epileptic syndromes in first-degree relatives. Diagnostic evaluation was made according to the classification of the International League Against Epilepsy [Commission on Classification, 1989], and the analysis of records (comprising EEGs) from previous hospitalizations. All patients were directly interviewed, and the records analyzed, by one of us (P.T.), and all electroclinical data were independently reviewed by another epileptologist (Dr. Dravet, Marseille). The control group included blood donors with no personal history of epilepsy or seizure. To minimize ethnic heterogeneity, both groups were entirely of Caucasian origin and their families were of central, eastern, or southern French extraction. The design of the study was approved by the Paris Hospitals Ethics Committee.

The mean ages of the patient and control groups were 28.3 years and 37.2 years, respectively. The sex ratios (female:male) in the two groups were 1.72:1 and 1.55:1, respectively.

Polymorphism Analysis

We analyzed dinucleotide repeat polymorphisms located in the GABRA5 and GABRB3 genes by PCR amplification, using the primers and conditions previously described by Mutirangura et al. [1992] and by Glatt et al. [1992]. Genotyping was conducted blind to diagnostic status.

Statistical Analysis

Two different strategies were used in the statistical analysis of the data. The first was to examine each marker independently. The association between affection status (case or control) and a particular allele, say A1, can be assessed by counting the numbers of A1 and non-A1 alleles in the two groups, and presenting the numbers in a 2*2. The difference of A1 frequencies between cases and controls were then tested by a chi-square test with Yates and Bonferroni corrections.

The second strategy was to test whether there was an overall association between the disorder and all of the alleles at the locus, before testing for association with individual alleles. Denoting the alleles by A1, A2, . . . , An, we counted the total numbers in a n*2 table. A chi-square test of homogeneity could then be used.

RESULTS

Eighty-eight cases and one hundred control subjects were genotyped at the GABRA5 and GABRB3 loci. The alleles, their sizes, and their frequencies are reported in Tables I and II. Except for a new rare allele of 135 bp at the GABRA5 locus, the patterns are in accordance with those reported by Mutirangura et al. [1992] (for GABRB3) and Glatt et al. [1992] (for GABRA5). No evidence for association between JME and the two candidate genes was observed either by testing each allele separately with correction for multiple testing, or by testing all alleles simultaneously by a chi-square test of homogeneity (Tables I and II).

DISCUSSION

Given the central place of the GABAergic system in current etiopathogenic theories concerning epilepsies, and the location of the GABRA5 and GABRB3 genes in the regions of the human genome implicated in diseases with epilepsy, it was reasonable to consider GABRA5 and GABRB3 as good candidate genes. This study provides evidence against an association between the GABRA5 and GABRB3 loci and JME, the most common IGE.

This negative result should nevertheless be interpreted with caution, given the possible heterogeneity of JME. GABRA5 and GABRB3 genes could be associated with JME in other populations, either because of their

TABLE I. Comparison of GABRB3 Allele Frequency Between JME and Control Subjects*

Alleles, GABRB3	Frequency of alleles: Cases (alleles = 176) (%)	Frequency of alleles: Controls (alleles = 200) (%)	χ^2 on 1 df	*P*
1 (201bp)	1 (0.57)	3 (1.5)	0.124	NS
2 (199bp)	5 (2.84)	6 (3.0)	0.000	NS
3 (197bp)	22 (12.50)	17 (8.50)	1.012	NS
4 (195bp)	4 (2.27)	8 (4.0)	0.380	NS
5 (193bp)	29 (16.48)	26 (13.0)	0.558	NS
6 (191bp)	5 (2.84)	11 (5.50)	0.946	NS
7 (189bp)	6 (3.41)	5 (2.5)	0.064	NS
8 (187bp)	9 (5.11)	16 (8.00)	0.731	NS
9 (185bp)	17 (9.66)	17 (8.50)	0.007	NS
10 (183bp)	15 (8.52)	13 (16.5)	0.165	NS
11 (181bp)	63 (36.79)	78 (39.0)	0.131	NS

*Testing each allele separately; df, degree of freedom; NS, nonsignificant: bp, base pairs. Testing all alleles simultaneously: $\chi^2 = 6.73$, df = 10, *P* = NS.

TABLE II. Comparison of GABRA5 Allele Frequency Between JME and Control Subjects*

Alleles, GABRA5	Frequency of alleles: Cases (alleles = 172) (%)	Frequency of alleles: Controls (alleles = 200) (%)	χ^2 on 1 df	P
1 (149bp)	3 (1.75)	3 (1.5)	0.000	NS
2 (147bp)	28 (16.28)	19 (9.50)	3.260	NS
3 (145bp)	24 (13.95)	27 (13.5)	0.000	NS
4 (143bp)	48 (27.91)	57 (28.5)	0.000	NS
5 (141bp)	51 (29.65)	66 (33.0)	0.338	NS
6 (139bp)	5 (2.91)	14 (7.00)	2.400	NS
7 (137bp)	9 (5.23)	10 (5.00)	0.000	NS
8 (135bp)	4 (2.32)	4 (2.0)	0.000	NS

*Testing each allele separately; df, degree of freedom; NS, nonsignificant; bp, base pairs. Testing all alleles simultaneously; $\chi^2 = 6.841$, df = 7, P = NS.

different geographical origin or because of the existence of different associated clinical attributes (e.g., presence or absence of GTCS or absence seizures). Larger samples will be needed to test this last possibility.

Second, failure to demonstrate linkage disequilibrium by conventional methods does not imply its absence [Thompson et al., 1988]. Indeed, it has been shown that very large sample sizes would be required to demonstrate negative linkage disequilibrium [Thompson et al., 1988]. Since we are unable to determine the frequency of a putative susceptibility GABRA5 or GABRB3 variant in the population we studied, and the type of the eventual linkage disequilibrium (negative or positive), we must consider the possibility that our sample size is not large enough to detect disequilibrium. Nevertheless, we could conclude that, in case of positive disequilibrium, our sample is sufficient to detect it if it exists, according to the estimates provided by Thompson et al. [1988].

The third possibility is that the physical distances between the markers in the GABRA5 and GABRB3 genes we used and the putative responsible variant are too large to allow detection of linkage disequilibrium. However, GABRA5 and GABRB3 genes are not large enough to make this hypothesis likely [Sinnett et al., 1993].

In conclusion, we can find no evidence for the association of JME with the GABRA5 and GABRB3 genes. However, it would be valuable to study larger samples, to determine whether candidate genes are associated with JME subgroups, defined according to the presence or absence of GTCS or absence seizures, or whether an eventual negative linkage disequilibrium exists.

ACKNOWLEDGMENTS

This work was supported by grants from the Association Française contre les Myopathies, the Groupement d'Etude et de Recherche sur les Génomes, the Centre National de la Recherche Scientifique, the Institut National de la Recherche Médicale, and the Fond National Suisse pour la Recherche.

REFERENCES

Aguglia U, Gambardella D, Concolino D, Porta G, Sirchia S, Quattrone A (1995): Severe epilepsy in a patient with Prader-Willi syndrome due to a inversion-duplication of chromosome 15q11–q13: A clinical, neurophysiological, and molecular genetic study. 21st International Epilepsy Congress. Sydney, Australia, September 3–8.

Anderson VE, Hauser WA, Olaffson E, Rich S (1990): Genetic aspects of the epilepsies. In Sillanpaa M, Johanssen SI, Blennow G. Dam M (eds): "Paediatric Epilepsy." Petershield, UK: Wrightson Biomedical, pp 37–56.

Buckle VJ, Fujita N, Ryder-Cook AS, et al. (1989): Chromosomal localization of $GABA_A$ receptor subunit genes: Relationship to human genetic disease. Neuron 3:647–654.

Bundey S, Hardy C, Vickers V, Kilpatrick MW, Corbett JA (1994): Duplication of 15q11–q13 region in a patient with autism, epilepsy and ataxia. Dev Med Child Neurol 36:736–742.

Clerget-Darpoux F, Babron M-C, Bonaïti-Pellié C (1990): Assessing the effect of multiple linkage tests in complex diseases. Genet Epidemiol 7:245–253.

Commission on Classification and Terminology of the International League Against Epilepsy (1989): A revised proposal for the classification of epilepsy and epileptic syndromes. Epilepsia 30:268–278.

Delgado-Escueta AV, Enrile Bascal F (1984): Juvenile myoclonic epilepsy of Janz. Neurology 34:285–294.

Delgado-Escueta AV, Greenberg D, Weissbecker K (1990): Gene mapping in the idiopathic generalized epilepsies: Juvenile myoclonic epilepsy, childhood absence epilepsy, epilepsy with grand mal seizures, early childhood myoclonic epilepsy. Epilepsia 31[Suppl]:19–29.

Durner M, Sander T, Greenberg DA, Johnson K, Beck-Mannagetta G, Janz D (1991): Localization of idiopathic generalized epilepsy on chromosome 6p in families of juvenile myoclonic epilepsy. Neurology 41:1651–1655.

Elmslie FV, Williamson MP, Rees M, Whitehouse WP, Kerr M, Juel Kjeldsen M, Pang KA. Sundqvist A, Richens A, Friis M, Chadwick D, Gardiner RM (1995): Linkage analysis in nineteen families between juvenile myoclonic epilepsy and marker loci on chromosome 6p provides no evidence for a locus predisposing to JME in this region. "Concerted Action. Workshop III: Genetic Linkage Analysis of Inherited Epilepsies, Abstract Book." pp 9–10.

Glatt KA, Sinnett D. Lalande M (1992): Dinucleotide repeat polymorphism at the $GABA_A$ receptor $\alpha 5$ (GABRA5) locus at chromosome 15q11–q13. Hum Mol Genet 1:348.

Greenberg DA, Delgado-Escueta AV, Maldonado HM, Widelitz H (1988a): Segregation analysis of juvenile myoclonic epilepsy. Genet Epidemiol 5:81–94.

Greenberg DA, Delgado-Escueta AV, Widelitz H, Sparkes RS, Treiman L, Maldonado HM, Park MS, Terasaki PI (1988b): Juvenile myoclonic epilepsy (JME) may be linked to the BF and HLA loci on human chromosome 6. Am J Med Genet 31:185–192.

Grunewald RA, Panayiotopoulos CP (1993): Juvenile myoclonic epilepsy. A review. Arch Neurol 50:594–598.

Janz D (1989): Juvenile myoclonic epilepsy. Cleve Clin J Med 56:23–33.

Janz D, Durner M, Beck-Mannagetta G, Pantazis (1989): Family studies on the genetics of juvenile myoclonic epilepsy (epilepsy with impulsive petit mal). In Beck-Mannagetta G, Anderson VE, Doose H, Janz D (eds): "Genetics of the Epilepsies." Heidelberg: Springer-Verlag. pp 43–52.

Leppert M, Anderson VE, Quattlebaum T, Stauffer D, O'Connell P, Nakamura Y, Lalouel J-M, White R (1989). Benign familial neonatal convulsions linked to genetic markers on chromosome 20. Nature 337:647–648.

Lewis TB, Leach RJ, Ward K, O'Connell P, Ryan SG (1993): Genetic heterogeneity in benign familial neonatal convulsions: Identification of a new locus on chromosome 8q. Am J Hum Genet 53:670–675.

Liu A, Degado-Escueta AV, Weissbecker K, Serratosa S, Treiman LJ, Sparkes RS (1992): Chromosome 6p markers and juvenile myoclonic epilepsy: Negative linkage and evidence for a second EJM locus. Epilepsia 33:73.

Meldrum BS (1975): Epilepsy and GABA-mediated inhibition. Int Rev Neurobiol 17:1–36.

Mutirangura A, Ledbetter SA, Kuwano A, Chinault AC, Ledbetter DH (1992): Dinucleotide repeat polymorphism at the $GABA_A$ receptor $\beta 3$ (GABAB3) locus in the Angelman/Prader-Willi region (AS/PWS) of chromosome 15. Hum Mol Genet 1:67.

Panayiotopoulos CP, Obeid T (1989): Juvenile myoclonic epilepsy: An autosomal recessive disease. Ann Neurol 25:440–443.

Risch N, Merikangas K (1996): The future of genetic studies of complex human diseases. Science 273:1516–1517.

Sinnett D, Wagstaff J, Glatt K, Woolf E, Kirkness EJ, Lalande M (1993): High-resolution mapping of the γ-aminobutyric acid receptor subunit β3 and α5 gene cluster on chromosome 15q11–q13, and localization of breakpoints in two Angelman syndrome patients. Am J Hum Genet 52:1216–1229.

Thompson EA, Deebs F, Walker D, Motulsky AG (1988): The detection of linkage disequilibrium between closely linked markers: RFLPs at the AI-CIII apolipoprotein gene. Am J Hum Genet 42:113–124.

Tsuboi T, Christian W (1973): On the genetics of primary generalized epilepsy with sporadic myoclonias of impulsive petit mal type. Humangenetik 19:155–182.

Weissbecker KA, Durner M, Janz A, Scaramelli A, Sparkes RS, Spence MA (1991): Confirmation of linkage between juvenile myoclonic epilepsy locus and the HLA region of chromosome 6. Am J Med Genet 38:32–38.

Whitehouse WP, Rees M, Curtis D, Sundqvist A, Parker K, Chung E, Baralle D, Gardiner M (1993): Linkage analyzed of idiopathic generalized epilepsy (IGE) and marker loci on chromosome 6p in families of patients with juvenile myoclonic epilepsy: No evidence for an epileptic locus in the HLA region. Am J Hum Genet 53:652–662.